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(71) Applicant *(for all designated States except US)*: EUGENE SCIENCE INC. [KR/KR]; 5th floor, DBM Building, 200–30 Dongkyo–dong, Mapo–gu, Seoul 121–200 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NOH, Seung–Kwon [KR/KR]; 2–56 Sambu Apt., 30–3 Yeoido–dong, Youngdengpo–gu, Seoul 151–010 (KR). CHUNG, Dae–Won [KR/KR]; 104–205 Dusandonga Apt., Kwonsun–dong, Kwonsun–gu, Suwon 441–390 (KR).

(74) Agent: LEE, Han–Young; 8th floor, Seowon Building, 1675–1, Seocho–dong, Seocho–gu, Seoul 137–070 (KR).

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(54) Title: WATER–SOLUBLE STEROL DERIVATIVE FOR INHIBITING CHOLESTEROL ABSORPTION AND PROCESS FOR PREPARING THE SAME

(57) Abstract

The present invention relates to water–soluble sterol derivatives for inhibiting cholesterol absorption and process for preparing the same. The process for preparing the phytosterol derivatives of the present invention comprises the steps of: obtaining intermediate compounds by reacting phytosterols and succinic or glutaric anhydride in a non–polar solvent in the presence of a basic catalyst; and, coupling the intermediate compounds with hydrophilic polymers in a non–polar organic solvent in the presence of a basic catalyst and a coupling agent. The phytosterol derivatives of the invention may be incorporated directly in water–based foodstuffs (e.g., beverages) in any conventional manner.

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WATER-SOLUBLE STEROL DERIVATIVE FOR INHIBITING CHOLESTEROL ABSORPTION AND PROCESS FOR PREPARING THE SAME

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to water-soluble sterol derivatives for inhibiting cholesterol absorption and process for preparing the same.

Description of the Prior Art

It has been well known that cholesterol, when taken excessively, may cause cardiovascular disease. Without a low cholesterol diet, it is difficult to reduce the risk of cardiovascular disease resulting from high cholesterol consumption. While medicines have been developed for hyperlipidemia, they have side effects such as hepatic disorders resulting from inhibition of cholesterol synthesizing enzyme, thereby limiting their use as means for reducing serum cholesterol levels in human.

Numerous materials have been reported as having the effect of lowering serum cholesterol levels in animal bodies. For instance, chitosan, phytosterol, inositol and pectin have been used for the purpose of reducing serum cholesterol levels. Of these materials, phytosterols, which originate from plants, are believed to lower serum cholesterol levels, particularly LDL-cholesterol levels, by inhibiting absorption of cholesterol in the intestine through competition with cholesterol. It is believed that phytosterols do not significantly affect the biosynthesis of cholesterol or have significant side effects.

Phytosterols are steroid alcohols found in higher plant and include stigmasterol, spinasterol, campesterol and sitosterol, which has α, β and γ-types. Among various

phytosterols, the cholesterol lowering effect of β-sitosterol(24-ethyl-5α-cholestene-3β-ol) has been demonstrated in animal studies, as well as in clinical tests(see: Sugano, M. et al., *J. Nutr.*, 107:2011-2019, 1977). β-sitosterol ester compounds generated from a substitution of fatty acid have also been reported as having a cholesterol lowering effect similar to that of β-sitosterol(see: Mattson, F. H. et al., *J. Nutr.*, 107:1139-1146, 1977). For example, when 2 grams of β-sitosteryl oleate were dosed to adult humans for 5 days, cholesterol absorption was reduced by about 33%(see: Mattson, F. H. et al., *Am. J. Clin. Nutr.*, 35:697-700, 1982). In addition to cholesterol lowering effect, β-sitosterol is known to be the major component of *Zea mays L.* which is used for treating gingivitis and alveolitis.

Despite their cholesterol-lowering effect, phytosterols have not been in wide use,since phytosterols are not soluble in water or in oil, their use as food additives for reducing serum cholesterol limiting levels. That is, due to their hydrophobic and lipophobic properties, phytosterols have been formulated primarily in tablet or capsule form.

In the past, various phytosterol compounds that are soluble in oil have been developed in the art(see: U.S. Patent No. 5,502,045; Hartman, L. *Chem. Rev.*, 58:845-864, 1958; Mattson, F. H. et al., *J. Lipid Res.*, 5:374-377, 1964; and Korean Patent laid-open publication No. 99-74136). While these compounds are soluble in oil, they are insoluble in water and are not thus suitable for use as cholesterol-lowering additives for water-based food products.

On the other hand, polyoxyethylene phytostanol ether compounds have been used as an emulsifier for cosmetics(see: U.S. Patent Nos. 5,846,458, 5,593,622 and 5,676,971). Among these compounds, phytosterols are coupled to polyethylene glycols(PEGs) by relatively permanent ether bonds. As a result, while the compounds

are soluble in water, owing to their ether bonds, phytosterol moieties may not become released from PEGs when administered in the body. As a result, these compounds have been used primarily for cosmetic applications, particularly for skin and hair care products, but still not for food additives.

U.S. Patent No. 5,932,562 discloses compositions for water-based food additives for reducing cholesterol absorption. The compositions include an aqueous based micellar mix which is dried to provide a mixture of finely divided plant sterol and lecithin. Because the mixture is in micellar form, its use as food additives is believed to be significantly restricted.

U.S. Patent No. 5,880,131 discloses a compound having taxols coupled to polyethylene glycols with a molecular weight ranging from 20,000 to 80,000 for the purpose of making water soluble taxol derivatives. While the resulting compound is soluble in water, it is not suitable for use as cholesterol-reducing food additives.

Therefore, there are strong reasons for exploring and developing water-soluble sterol derivatives for wide and convenient use as cholesterol-reducing food additives and/or supplements.

SUMMARY OF THE INVENTION

The present inventors have made an effort to solve the said problems, and prepared novel water-soluble sterol derivatives for inhibiting intestinal cholesterol absorption by reacting phytosterol with succinic anhydride or glutaric anhydride to obtain intermediate compounds, and coupling the intermediate compounds with hydrophilic polymer to prepare the water-soluble sterol derivatives, thereby phytosterol moieties can be released from the hydrophilic polymer in the intestine.

A primary object of the present invention is, therefore, to provide water-soluble sterol derivatives for inhibiting intestinal cholesterol absorption.

The other object of the invention is to provide a process for preparing the water-soluble sterol derivatives by reacting phytosterol with succinic anhydride or glutaric anhydride, and coupling with hydrophilic polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and the other objects and features of the present invention will become apparent from the following descriptions given in conjunction with the accompanying drawings, in which:

Figure 1 is a reaction scheme for the intermediate compound of the present invention.

Figure 2 is ^{1}H-NMR spectrum of phytosterol derivative prepared by the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides water-soluble sterol derivatives for inhibiting intestinal cholesterol absorption. In particular, the sterol derivatives have phytosterols coupled to hydrophilic polymers by chemical bonds that can be hydrolyzed by enzymes in the intestine when the phytosterol derivatives are orally administered in animals. In other words, the chemical bonds between the phytosterols and the hydrophilic polymers are biodegradable in the intestine by enzymes present therein such that phytosterol moieties can be released from hydrophilic polymer moieties for inhibiting intestinal absorption of cholesterol. While various types of chemical bonds are

usable for the phytosterol derivatives of the present invention, ester and/or amide bonds are preferably employed.

The chemical nature of the phytosterol derivatives of the present invention is illustrated and described hereinbelow.

Various types and forms of phytosterol, including phytostanols, may be used for the phytosterol derivatives of the present invention. Accordingly, in describing the water-soluble sterol derivative of the invention, the term "phytosterols" is employed to mean all types, forms and/or mixtures of phytosterols and phytostanols such as stigmasterol, spinasterol, campesterol, sitosterol, sitostanol and campestanol; and, the term "hydrophilic polymers" is employed to mean water-soluble carriers that can bond with the intermediate compounds by chemical bonds capable of being hydrolyzed(i.e., biodegradable) by enzymes in the intestine when the resultant compounds are orally administered in animals, such as polyethylene glycol(PEG), xanthan gum, guar gum, sodium alginate, dextrin, oligosaccharide, chitosan and carboxymethylcellulose(CMC). In this regard, the hydrophilic polymers are provided for illustration purposes only and are not meant to limit the scope of the present invention. Accordingly, while any of the foregoing hydrophilic polymers can be used, PEGs having an average molecular weight ranging from 500 to 4,000, preferably from 1,000 to 3,000, and more preferably from 1,000 to 2,000 are suitable for use as hydrophilic polymers.

The process for preparing the phytosterol derivatives of the present invention comprises the steps of: obtaining intermediate compounds by reacting phytosterols and succinic or glutaric anhydride in a non-polar solvent in the presence of a basic catalyst; and, coupling the intermediate compounds with hydrophilic polymers in a non-polar organic solvent in the presence of a basic catalyst and a coupling agent.

The process for preparing the water-soluble phytosterol derivatives of the present invention is described in more detail by the following steps.

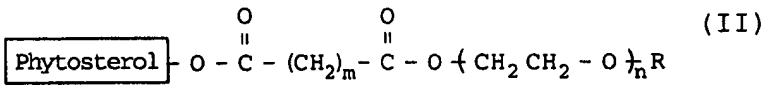
Step 1: Preparation of Intermediate Compounds(I)

Phytosterol and succinic or glutaric anhydride are dissolved in a non-polar solvent in a molar ratio of 1:1.0 to 1:1.5 and more preferably 1:1.3, a basic catalyst is subsequently added, and heated to a temperature preferably ranging from 40 to 150℃ and more preferably to the boiling temperature of the non-polar organic solvent preferably for 2 to 20 hours and more preferably for 4 to 9 hours. While toluene, methylene chloride, dichloroethane, tetrahydrofuran, benzene and diethylether are preferably employed as the non-polar organic solvent, other non-polar solvents may be used; and, 4-dimethylaminopyridine(DMAP), pyridine and triethylamine may be used as the basic catalyst. After the starting materials disappear in TLC, the non-polar solvent is evaporated from the reaction mixture to give a solid residue. The solid residue is re-dissolved in methylene chloride or dichloroethane, washed with distilled water to extract impurities, dried over anhydrous $MgSO_4$, filtered, and cooled to about 4℃ for about 12 hours so as to crystalize unreacted succinic or glutaric anhydride. After removing the crystalized succinic or glutaric anhydride by filtration, the solvent of the filtrate is then evaporated, to obtain intermediate compounds(I)(see: Figure 1).

Step 2: Preparation of Water-Soluble Phytosterol Derivatives

The intermediate compounds(I) obtained in Step 1 and a hydrophilic polymer such as PEG, are dissolved in a non-

polar organic solvent in the presence of basic catalyst in a molar ratio ranging from 1:1 to 2:1. To the reaction mixture, a coupling agent are added, stirred at room temperature for about 5 to 15 hours, then filtered to remove byproduct. In this step, the non-polar organic solvent and basic catalyst used in Step I may be employed, and 1,3-dicyclohexylcarbodiimide(DCC), 1-3-diisopropylcarbodiimide(DIPC), 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide, oxalyl chloride, carbonyl diimidazole, 2-chloropyridium, 2,2'-dipyridyl disulfide and 2-imidazoyl disulfide are preferably used as the coupling agent. The filtrate thus obtained is then precipitated in n-hexane, filtered and washed with n-hexane. Finally, water-soluble phytosterol derivatives are prepared by vacuum-drying the filtrate. The chemical structure of the phytosterol derivatives of the present invention is represented as followings:

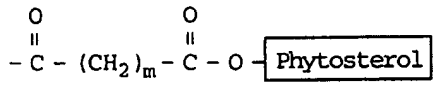


(II)

wherein,

n is an integer ranging from 22 to 90; and,

R is selected from the group consisting of -H, $-CH_3$ and



(wherein, m is 2 or 3).

The cholesterol lowering effect of the phytosterol derivatives of the present invention was investigated by employing experimental animals. The study demonstrated that the phytosterol derivatives have a cholesterol-absorption inhibiting effect as will be discribed in

greater detail in Examples. A study undertaken to investigate the solubility of the phytosterol derivatives also demonstrated that the phytosterol derivatives are soluble in water.

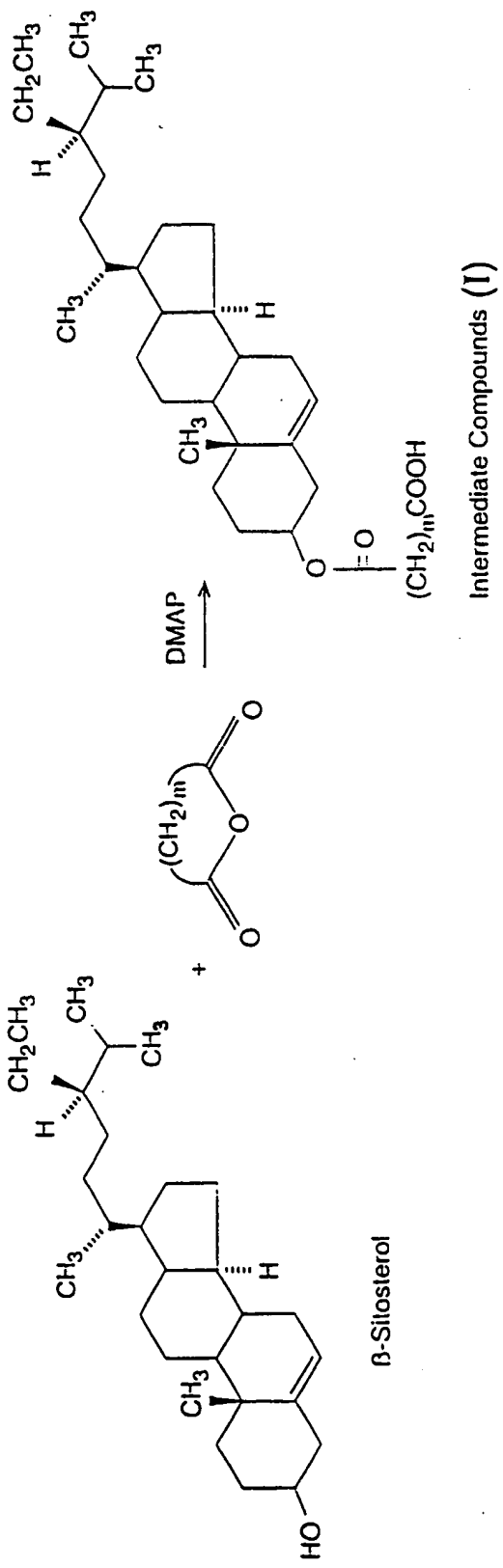
Accordingly, the phytosterol derivatives of the present invention may be incorporated directly in water-based foodstuffs(e.g., beverages) in any conventional manner.

The present invention is further illustrated in the following examples, which should not be taken to limit the scope of the invention.

Example 1: Preparation of Intermediate Compounds

Example 1-1

10g of β-sitosterol and 55.2g of succinic anhydride(1:1.15 in a molar ratio) are dissolved in toluene in a round-bottomed flask with reflux tube and dean-stark. To the reaction mixture was added 2.4g of DMAP, then refluxed for 6 hours. After confirming the completion of the reaction with thin layer chromatography(TLC), the solvent was evaporated from the reaction mixture. The remaining solid residue was dissolved in methylene chloride and washed with distilled water. The methylene chloride layer was then kept under refrigeration at 4℃ for 14 hours so as to crystalize unreacted succinic anhydride. After removing the crystalized succinic anhydride by filtration, the filtrate was evaporated, and obtained an intermediate compound(I, m=2). The yield of the intermediate compound was 70%.


β-Sitosterol
DMAP
Intermediate Compounds (I)

Example 1-2

The intermediate compound were prepared in an analogous manner as in Example 1-1 except for using β-sitosterol and succinic anhydride in a molar ratio of 1:1.20 and refluxing for 4 hours. The yield of the intermediate compound(I, m=2) was 73%.

Example 1-3

10g of β-sitosterol and 65.7g of glutaric anhydride (1:1.15 in a molar ratio) were dissolved in toluene in a round-bottomed flask with reflux tube and dean-stark. To the reaction mixture was added 2.4g of DMAP, then refluxed for 10 hours. After confirming the completion of the reaction with thin layer chromatography(TLC), the solvent was evaporated from the reaction mixture. The remaining solid residue was dissolved in methylene chloride and was washed with distilled water. The organic layer was then left to stand at 4℃ for 14 hours so as to crystalize unreacted glutaric anhydride. After removing the crystalized glutaric anhydride by filtration, the filtrate was evaporated to give an intermediate compound(I, m=3). The yield of the intermediate compound was 62%.

Example 2: Preparation of Water-Soluble Phytosterol Derivative

5g of PEG having a molecular weight of 2,000, 0.85g of DCC and 0.09g of DMAP were dissolved in a small amount of methylene chloride at room temperature. 1.93g(1.5 times the moles of PEG) of the intermediate compound(I, m=2) obtained in Example 1-1 was pre-dissolved in methylene chloride and was then added in a dropwise at 30℃ to the PEG/DCC/DMAP mixture. After 14 hours of stirring at 30℃, the mixture was filtered to remove dicyclohexylurea. The

filtrate was then precipitated in n-hexane, filtered and washed with n-hexane. 3.7g of water-soluble β -sitosterol derivative was given after vacuum drying the filtrate.

The structure of the β -sitosterol derivatives was confirmed by ^{1}H-NMR in $CDCl_3$, whose spectrum is disclosed in Figure 2. The ^{1}H-NMR spectrum confirmed that β -sitosterol was coupled to PEG by an ester bond. The degree of substitution(DS) of the β -sitosterol derivatives, which is defined as the average molar number of sterol moieties per one molecule of phytosterol derivatives, was determined to be about 1.09, based on the ^{1}H-NMR spectrum and the following equation:

$$DS = n*c/(a+b)$$

wherein,

n is the degree of polymerization of PEG; and,

a, b and c are the integrations of the peaks assigned in Figure 2.

Example 3: Optimization of Reaction Conditions

Example 3-1:

To determine the effect of the molecular weight of PEG on the degree of substitution, various phytosterol derivatives were prepared by employing PEGs having different molecular weights under the reaction conditions described in Example 2. The degree of substitution of the phytosterol derivatives(II) was measured in an analogous manner as in Example 2, whose results are summarized in Table 1 below.

Table 1:

Molecular Weight of PEG	Quantity of PEG(g)	PEG to Intermediate Compound (Molar Ratio)	Reaction Time (hrs)	Degree of Substitution
1,000	5	1 : 1.2	10	1.02
1,500	5	1 : 1.2	12	1.05
2,000	5	1 : 1.5	14	1.09
4,000	5	1 : 1.2	14	1.08

As clearly demonstrated in Table 1, the molecular weight of PEG does not have a significant effect on the degree of substitution of the phytosterol derivatives of the present invention.

Example 3-2:

The solubility of the phytosterol derivatives of the present invention was measured by dissolving various samples in water and leaving to stand the mixture for 2 days at different temperatures, whose results are shown in Table 2 below.

Table 2:

Molecular Weight of PEG	Degree of Substitution	Temperature (℃)	Maximum Concentration (wt%)	
			Basis of Phytosterol Derivatives	Basis of Sterol Moiety(g)
1,000	1.02	35	10.0	3.4
1,000	1.02	RT*	8.0	2.7
1,000	1.02	4	8.0	2.7
1,500	1.05	35	10.0	2.7
1,500	1.05	RT*	8.0	2.2
1,500	1.05	4	8.0	2.2
2,000	1.09	35	13.2	3.0
2,000	1.09	RT*	10.0	2.2
2,000	1.09	4	10.0	2.2
4,000	1.08	35	14.0	1.8
4,000	1.08	RT*	14.0	1.8
4,000	1.08	4	14.0	1.8

*: RT(room temperature)

As clearly demonstrated in Table 2, the phytosterol derivatives prepared with PEG having a molecular weight 1,000, 1,500, 2,000 and 4,000(hereinafter referred to as "phytosterol derivatives of 1,000, 1,500, 2,000 and 4,000 PEG", respectively) are soluble in water. While the phytosterol derivatives of 4,000 PEG is more soluble in water than the phytosterol derivatives of 1,000, 1,500 and 2,000 PEG on the basis of weight concentration of phytosterol derivatives, the phytosterol derivatives of 1,000, 1,500 and 2,000 PEG are more soluble in water than the phytosterol derivatives of 4,000 PEG on the basis of sterol moiety.

Example 3-3:

PEG having a molecular weight of 2,000 was reacted with the intermediate compound obtained in Example 1-1 under the reaction conditions analogous to those described in Example 2 with the exception of the quantity of PEG. Phytosterol derivatives with variable degrees of substitution were prepared in large-scale, whose results are shown in Table 3 below. As clearly demonstrated in Table 3, the scale of phytosterol derivative production(i.e., the amount of PEG) is not believed to have a significant effect on the degree of substitution(DS) of phytosterol derivatives.

Table 3:

Phytosterol Derivative Samples	Quantity of PEG (g)	PEG to Intermediate Compound (Molar ratio)	Reaction Time (hrs)	DS*	Quantity of Phytosterol Derivatives (g)	Yield** (%)
1	30	1 : 1.5	14	1.09	24.4	63.4
2	30	1 : 1.5	14	0.98	28.2	75.1
3	100	1 : 1.5	18	0.92	86.2	69.7
4	100	1 : 1.5	23	1.33	86.7	64.6

*: DS, degree of substitution

**: Yield(%), moles of phytosterol derivatives/moles of PEG x 100(%)

Example 4: Solubility of Phytosterol Derivatives Depending on Temperature

Since the phytosterol derivatives of the present invention are suitable for use in "cold" beverages, which are stored under refrigeration, their solubility depending on temperature was studied. More particularly, the solubility of Samples 4 of Table 3 above in water was measured under the temperatures indicated in Table 4 below.

The solubility in water at 4°C was measured to be higher than 1.0 wt% based on the amount of sterol moieties(i.e., higher than 10mg of sterol moieties per 1 ml of water). It was also demonstrated that temperature does not have a significant effect on the solubility of the phytosterol derivatives.

Table 4:

Phytosterol Derivative Samples	Degree of Substitution	Temperature (°C)	Maximum Concentration(wt%)	
			Basis of Phytosterol Derivatives	Basis of Sterol Moieties
1	1.09	35	13.2	2.8
		RT*	10.0	2.2
		4	10.0	2.2
2	0.98	35	10.0	2.0
		RT*	10.0	2.0
		4	10.0	2.0
3	0.92	35	20.0	3.8
		RT*	20.0	3.8
		4	20.0	3.8
4	1.33	35	10.0	2.6
		RT*	10.0	2.6
		4	10.0	2.6

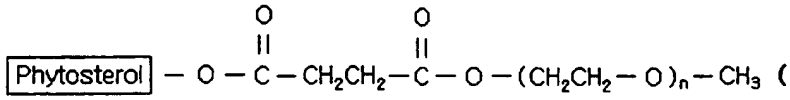
*: RT(room temperature)

The amount of sterol moieties generally recommended for reducing serum cholesterol levels is about 2.7 grams per day(see: Weststrate et al., *Eur. J. Clin. Nutr.*, 52:334-343, 1998; Johns et al., *Can. J. Physiol Pharmacol*, 75:217-227, 1997; Vanhanen et al., *Clin. Chim. Acta*, 205:97-107, 1992; Gylling et al., *J. Lip. Res.*, 37:1776-1785, 1996; Gylling et al., *Circulation*, 96:4226-4231, 1997; Gylling et al., *Diabetologia*, 37:773-780, 1994; Denke,

M.A., *Am. J. Clin. Nutr.*, 61:392-396, 1995; and Vanhanen et al., *Clin. Sci.*, 87:31-37, 1994). Since the solubility of Samples 4 in water at 4°C was measured to be more than 10 mg per 1ml of water based on the amount of sterol moieties, it would be possible to make a 300ml can of beverage containing 3.0g of sterol moieties with the phytosterol derivatives of the present invention.

Example 5: Preparation of Phytosterol Derivative(III) by Coupling Intermediate Compound(I) to Monomethyl Ether-PEG

Phytosterol derivatives(III) were prepared by using monomethyl ether-PEG(MME-PEG), one end of which is blocked by a methyl ether group: 5g of MME-PEG having a molecular weight of 2000, 0.68g of DCC and 0.07g of DMAP were dissolved in a small amount of methylene chloride at room temperature. 1.54g(1.2 times to the moles of MME-PEG) of the intermediate compound(I) obtained in Example 1-2 was pre-dissolved in methylene chloride and was then added in a dropwise at 30°C to the MME-PEG/DCC/DMAP mixture. After 12 hours of stirring at 30℃, the mixture was filtered to remove dicyclohexylurea(DCU). The filtrate was then precipitated in n-hexane, filtered and washed with n-hexane. Phytosterol derivatives(III) were obtained by vacuum-drying the filtrate, whose chemical structure is schematically illustrated below:



(III),

wherein,

n is an integer ranging from 22 to 90.

^{1}H-NMR spectrum of the phytosterol derivatives (III) demonstrated that all hydroxyl groups of MME-PEG were reacted with the intermediate compounds(I) and coupled with phytosterols. The degree of substitution of the phytosterol derivatives(III) is one, while the solubility was measured to be substantially identical to that of Sample 2, Table 4.

Example 6: Preclinical Test of Cholesterol-Lowering Effect

The effect of the phytosterol derivatives of Sample 4, Table 4 was studied in an animal test with β-sitosterol as a positive control. For this study, six-week-old SPF SD male rats were divided into six groups and were housed in a room with a 12-hour light-dark cycle with free access to tap water and food for one week for acclimatization(see: Table 5 below). After a week of acclimatization, the rats were not given any food or water from 9 AM to 4 PM and then were administered at 4 PM with 10mg of cold cholesterol suspended in 0.5ml of corn oil and/or 0.025mg of ^{14}C-cholesterol in 0.08ml of ethanol for three days as indicated in Table 5. When being administered with cholesterol, each of the rats in Groups 3 and 4 was also administered with the amount of phytosterol derivatives equal to three and five, respectively, times the total amount of the administered cholesterol(see: Table 5 below), while each of the rats in Groups 5 and 6 was administered with the amount of the phytosterol derivatives equivalent, in terms of sterol moiety, to three and five, respectively, times the total amount of the administered cholesterol(see: Table 5). The rats were not given any rations or water for the three hour period immediately following the cholesterol/β-sitosterol/phytosterol derivative administration. Subsequently, the rats were given free access to rations and water until 9 AM the next morning. On the third day of cholesterol administration, the rats were not fed overnight and then were sacrificed the next

morning. The rats were anaesthetized with diethylether, and 6ml of blood were collected from their hearts and centrifuged at 2000xg for 20 minutes. To quantify the ^{14}C-cholesterol in the blood, a 1.5ml supernatant(plasma) was taken and a 10 ml cocktail solution was added. Using a liquid scintillation counter, radioactivity was counted for two minutes for each Sample. All data specified in Table 6 were represented as mean ± SD, and the statistical analysis of data was carried out by the Student's *t*-test. The significance of a value was accepted when *P* was less than 0.01.

Table 5:

Groups	Background	Negative Control	Sitosterol (Positive Control)		Phytosterol Derivatives of Sample 4, Table 4	
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Amount of Cholesterol Administered in Rats by p.o.(per os)	10mg of cold cholesterol in 0.5ml corn oil	0.025 mg of ^{14}C-cholesterol in 0.08 ml of ethanol & 10 mg of cold cholesterol in 0.5 ml of corn oil				
Sort of Samples	-	-	β -sitosterol		Phytosterol Derivatives	
Amount of Samples by p.o.(per os)	-	-	30 mg in 1.0 ml of corn oil	50 mg in 1.67 ml of corn oil	30* mg in 1.2 ml of Distilled Water	50* mg in 2.0 ml of Distilled Water
Numbers of Rats	3	6	5	5	6	6

*: the amount of β -sitosterol moieties

The results of the study are summarized in Table 6 below. As clearly demonstrated in Table 6, β -sitosterol(positive control) reduces cholesterol absorption by about 30%. The phytosterol derivatives of the present invention also reduced cholesterol absorption by about 30% and is hence believed to be as effective as β -sitosterol in inhibiting intestinal cholesterol absorption.

Table 6:

Groups	Cpm	% of Inhibition
Group 1 (cold cholesterol)	47.3± 19.7	Not Applicable
Group 2 (^{14}C- & cold cholesterol)	74566.4± 4121.7[a]	Not Applicable
Group 3 (^{14}C- & cold cholesterol and 3x β -sitosterol)	51766.9± 6602.4*	31
Group 4 (^{14}C- & cold cholesterol and 5x β -sitosterol)	52086.5± 2587.2*	30
Group 5 (^{14}C- & cold cholesterol and 3x phytosterol derivatives of Sample 4, Table 4)	53848.7± 8738.2*	28
Group 6 (^{14}C- & cold cholesterol and 5x phytosterol derivatives of Sample 4, Table 4)	50019.4± 8234.8*	33

a: Values are shown as mean ± SD(n=5 or 6).

*: denotes significant differences, $p<0.01$, compared with the negative control(Group 2).

* * * *

Although the preferred embodiments of the present invention have been disclosed for illustrative purpose, those who are skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. A phytosterol derivative compound for inhibiting absorption of cholesterol in the body of an animal, which comprises: a phytosterol moiety; and, a hydrophilic polymer moiety coupled to said phytosterol moiety by a biodegradable bond so as to give a water-soluble compound.

2. The compound of claim 1, wherein said biodegradable bond is a bond which can be hydrolyzed by an enzyme in the body of the animal, whereby said phytosterol moiety can be released from said hydrophilic polymer moiety when said water-soluble compound is administered to the body of the animal.

3. The compound of claim 2, wherein said biodegradable bond is selected from the group consisting of an ester bond and an amide bond.

4. The compound of claim 3, wherein said hydrophilic polymer moiety is a material selected from the group consisting of polyethylene glycol, xanthan gum, guar gum, sodium alginate, dextrin, oligosaccharide, chitosan and carboxymethylcellulose.

5. The compound of claim 4, wherein said hydrophilic polymer moiety is polyethylene glycol having an average molecular weight ranging from 500 to 4,000.

6. The compound of claim 5, wherein said average molecular weight of polyethylene glycol ranges from 1,000 to 3,000.

7. The compound of claim 6, wherein said average molecular weight of polyethylene glycol ranges from 1,000 to 2,000.

8. The compound of claim 7, wherein said average molecular weight of polyethylene glycol is equal to 2,000.

9. The compound of claim 8, wherein said phytosterol moiety is a material selected from the group consisting of stigmasterol, spinasterol, campesterol, sitosterol, sitostanol and campestanol.

10. The compound of claim 9, wherein said phytosterol moiety is β -sitosterol.

11. The compound of claim 5, wherein said phytosterol moiety is a material selected from the group consisting of stigmasterol, spinasterol, campesterol, sitosterol, sitostanol and campestanol.

12. The compound of claim 11, wherein said phytosterol moiety is β -sitosterol.

13. The compound of claim 5, wherein said polyethylene glycol is monomethyl ether-polyethylene glycol having an average molecular weight equal to 2,000.

14. The compound of claim 1, wherein said hydrophilic polymer moiety is a material selected from the group consisting of polyethylene glycol, xanthan gum, guar gum, sodium alginate, dextrin, oligosaccharide, chitosan and carboxymethylcellulose.

15. The compound of claim 14, wherein said hydrophilic polymer moiety is polyethylene glycol having an average molecular weight ranging from 500 to 4,000.

16. The compound of claim 15, wherein said average molecular weight of polyethylene glycol ranges from 1,000 to 3,000.

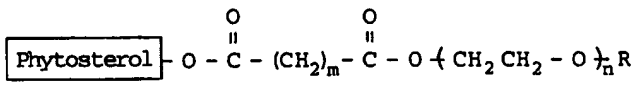
17. The compound of claim 16, wherein said average molecular weight of polyethylene glycol ranges from 1,000 to 2,000.

18. The compound of claim 17, wherein said average molecular weight of polyethylene glycol is equal to 2,000.

19. The compound of claim 18, wherein said phytosterol moiety is a material selected from the group consisting of stigmasterol, spinasterol, campesterol, sitosterol, sitostanol and campestanol.

20. The compound of claim 19, wherein said phytosterol moiety is β -sitosterol.

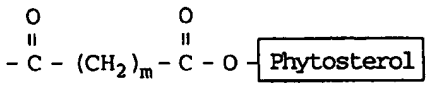
21. The compound of claim 1, wherein said water-soluble compound has the following formula:



wherein,

n is an integer ranging from 22 to 90; and,

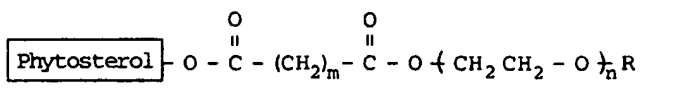
R is selected from the group consisting of -H, $-CH_3$ and



(wherein, m is 2 or 3).

22. The compound of claim 1, wherein said water-soluble compound is soluble in water by at least one weight percent on the basis of phytosterol moiety at a temperature ranging from 4 to 35°C.

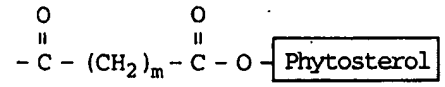
23. A phytosterol derivative compound which is represented as the following formula:



wherein,

n is an integer ranging from 22 to 90; and,

R is selected from the group consisting of -H, $-CH_3$ and



(wherein, m is 2 or 3).

24. The compound of claim 23, wherein said phytosterol is selected from the group consisting of stigmasterol, spinasterol, campesterol, sitosterol, sitostanol and campestanol.

25. The compound of claim 24, wherein said phytosterol is β-sitosterol.

26. A process for preparing a phytosterol derivative compound for inhibiting cholesterol absorption in the body of an animal, which comprises a step of coupling phytosterol with a hydrophilic polymer by a biodegradable bond so as to give a water-soluble compound.

27. The process of claim 26, wherein said biodegradable bond is a bond which can be hydrolyzed by an enzyme in the body of the animal, whereby said phytosterol can be released from said hydrophilic polymer when said water-soluble compound is administered to the body of the animal.

28. The process of claim 27, wherein said biodegradable bond is selected from the group consisting of an ester bond and an amide bond.

29. The process of claim 28, wherein said hydrophilic polymer is a material selected from the group consisting of polyethylene glycol, xanthan gum, guar gum, sodium alginate, dextrin, oligosaccharide, chitosan and carboxymethyl-cellulose.

30. The process of claim 29, wherein said hydrophilic polymer is polyethylene glycol having an average molecular weight ranging from 500 to 4,000.

31. The process of claim 30, wherein said average molecular weight of polyethylene glycol ranges from 1,000 to 3,000.

32. The process of claim 31, wherein said average molecular weight of polyethylene glycol ranges from 1,000 to 2,000.

33. The process of claim 32, wherein said average molecular weight of polyethylene glycol is equal to 2,000.

34. The process of claim 33, wherein said phytosterol is a material selected from the group consisting of stigmasterol, spinasterol, campesterol, sitosterol, sitostanol and campestanol.

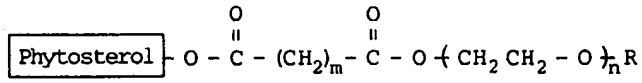
35. The process of claim 34, wherein said phytosterol is β -sitosterol.

36. The process of claim 30, wherein said phytosterol is a material selected from the group consisting of stigmasterol, spinasterol, campesterol, sitosterol, sitostanol and campestanol.

37. The process of claim 36, wherein said phytosterol is β -sitosterol.

38. The process of claim 30, wherein said polyethylene glycol is monomethyl ether-polyethylene glycol having an average molecular weight equal to 2,000.

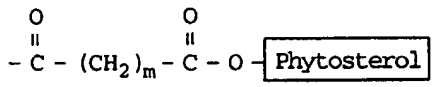
39. The process of claim 26, wherein said water-soluble compound has the following formula:



wherein,

n is an integer ranging from 22 to 90; and,

R is selected from the group consisting of -H, $-CH_3$ and



(wherein, m is 2 or 3).

40. The process of claim 26, wherein said coupling step comprises the steps of: reacting said phytosterol with a material selected from the group consisting of succinic anhydride and glutaric anhydride so as to obtain an intermediate compound; and, coupling said intermediate compound with said hydrophilic polymer so as to give said water-soluble compound.

41. The process of claim 40, wherein said phytosterol is reacted with said material in a nonpolar solvent in the presence of a basic catalyst.

42. The process of claim 41, wherein said intermediate compound is reacted with said hydrophilic

polymer in a nonpolar solvent in the presence of a basic catalyst and a coupling agent.

43. The process of claim 42, wherein said coupling agent is selected from the group consisting of 1,3-dicyclohexylcarbodiimide, 1-3-diisopropylcarbodiimide, 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide, oxalyl chloride, carbonyl diimidazole, 2-chloropyridium, 2,2'-dipyridyl disulfide and 2-imidazoyl disulfide.

44. The process of claim 43, wherein said basic catalyst is selected from the group consisting of 4-dimethylaminopyridine, pyridine and tri-ethylamine.

45. The process of claim 44, wherein said nonpolar solvent is selected from the group consisting of toluene, methylene chloride, dichloroethane, tetrahydrofuran, benzene and diethylether.

46. The process of claim 45, wherein said step of reacting said phytosterol with said material is performed using a molar ratio between said phytosterol and said material ranging from 1:1.0 to 1:1.5.

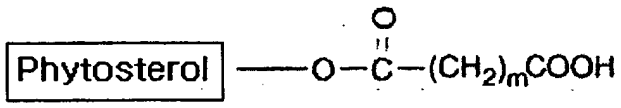
47. The process of claim 46, wherein said step of reacting said phytosterol with said material is performed for a time period ranging from 2 to 20 hours at a temperature ranging from 40 to 150°C.

48. The process of claim 47, wherein said step of reacting intermediate compound with hydrophilic polymer is performed using a molar ratio between said intermediate compound and said hydrophilic polymer ranging from 1:1 to 2:1.

49. The process of claim 48, wherein said step of reacting intermediate compound with hydrophilic polymer is

performed at room temperature for a time period ranging from 5 to 15 hours.

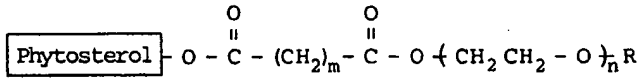
50. The process of claim 40, wherein said intermediate has the following formula:



wherein,

m is 2 or 3.

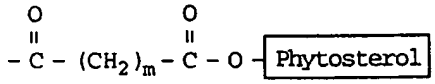
51. The process of claim 26, wherein said water-soluble compound has the following formula:



wherein,

n is an integer ranging from 22 to 90; and,

R is selected from the group consisting of -H, $-CH_3$ and



(wherein, m is 2 or 3).

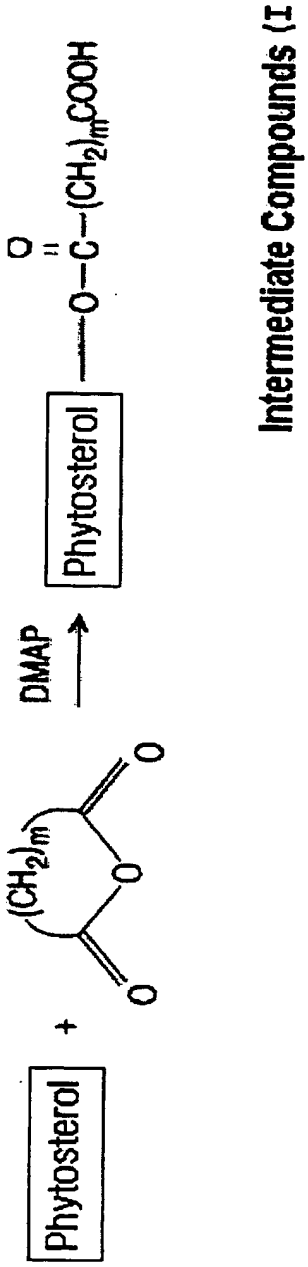

Intermediate Compounds (I
Phytosterol
+
DMAP
Phytosterol
O
O
O
O
(CH2)m
O=C—(CH2)mCOOH

Fig. 1

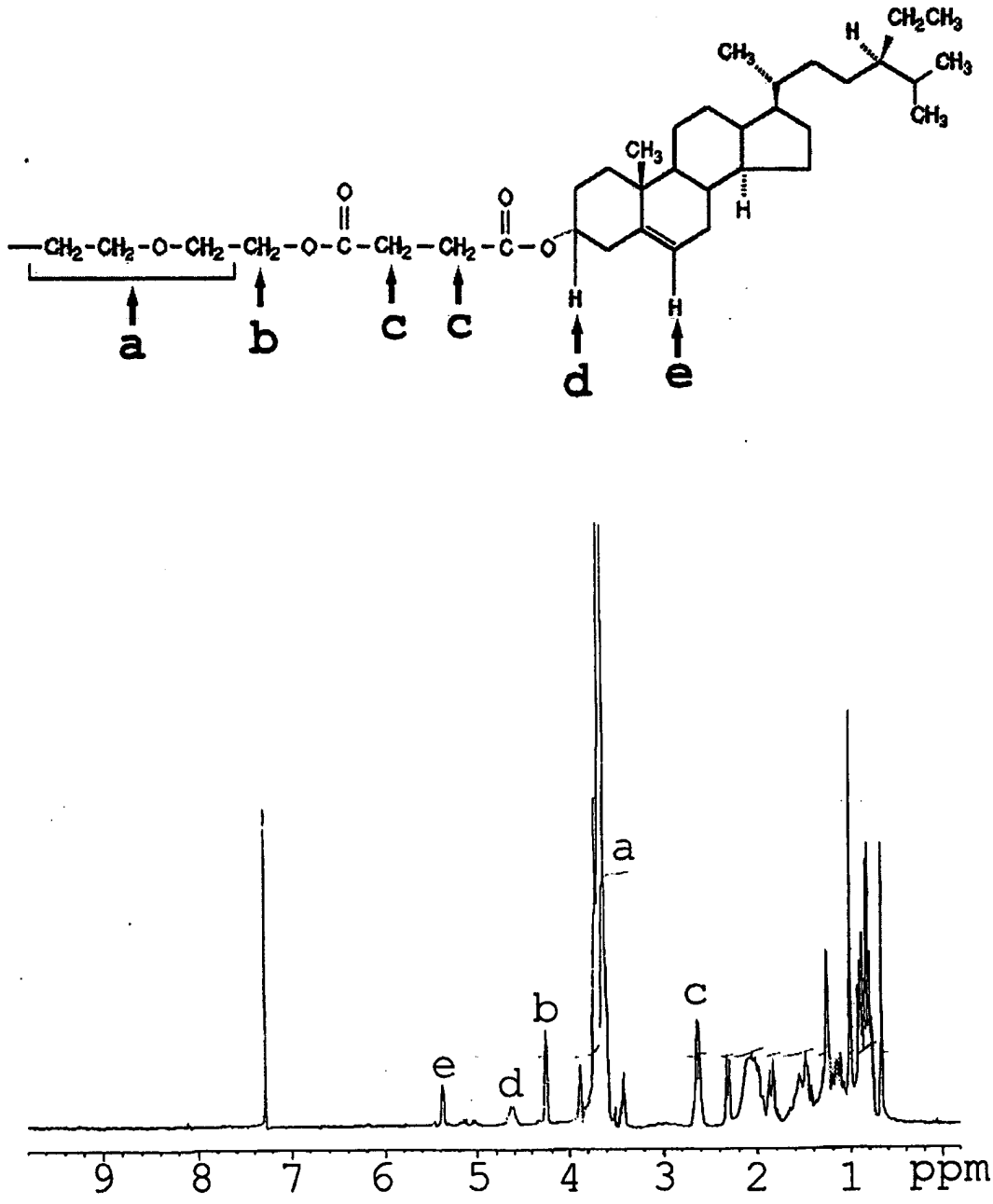


Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/00170

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07J 75/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC7 C07J

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)
CA registry, "phytosterol, water-soluble ", USPTO - US, EP, WO patent database, NCBI pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 938937 A page 1, line 1-31 page 2, line 1-29	1-51
A	Lipids, 1991, vol. 26, No. 3, pages 209-212: especially p209, line 1-19	1,3,26,28
A	JP 10-330422 A(Foundation for Scientific Technology Promotion, Japan)15 Dec 1998 (15.12.1998)	1,3,26,28
A	US 4183847 A (Arvind D. Deshmukh) 15 Jan 1980 (15.01.1980)	1,26
A	WO 9915546 A (RAISIO BBNECOL LTD) 1 Apr 1999 (01.04.1999) page 3,line 7-30 page 5, line 16-21 page 9, line 13-22	1, 2,26, 27 40 2, 27
A	Obshch. Khim. 1997, vol 47, No. 6, pages 1429-1430	40, 50

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 APRIL 2000 (26.04.2000)	02 MAY 2000 (02.05.2000)
Name and mailing address of the ISA/KR Korean Industrial Property Office Government Complex-Taejon, Dunsan-dong, So-ku, Taejon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer LIM, Hea Joon Telephone No. 82-42-481-5610

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/00170

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	J. Med. Chem. 1984, vol 27, No. 10, pages 1306-1312 especially p1306, p1309	1, 3, 26, 28, 40, 50

Form PCT/ISA/210 (continuation of second sheet) (July 1998)